REPORT DOCUMENTATION PAGE

AFRL-SR-BL-TR-98-0535

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the ... and maintaining the data needed and completing and reviewing the collection of information. Send comments regar... including suggestions for reducing the burden to Washington Headquarters Services, Directorate for Information Ope... VA 22202-4302 and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washing... 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2, 1998	Final 3/1/95-4/30/98

4. TITLE AND SUBTITLE OF REPORT Development of Corneal Equivalents for In Vitro Testing of Ocular Irritants

5. FUNDING NUMBERS Grant # F49620-95-1-0168

3312/A5

6. AUTHOR(S) Thomas E. Eurell

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Department of Veterinary Biosciences
College of Veterinary Medicine
University of Illinois
2001 S. Lincoln Ave., Urbana, IL 61802

8. PERFORMING ORGANIZATION REPORT NUMBER:

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFOSR/NL
110 Duncan Ave., B115
Bolling AFB, D.C., 20332-0001

10. SPONSORING/MONITORING AGENCY REPORT NUMBER:

11. SUPPLEMENTARY NOTES:

12a. DISTRIBUTION AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

Using tissue culture methods developed by our research team, we have achieved standardized methods for producing organotypic models of the rabbit and human cornea. One rabbit cornea will provide enough seed culture cells to produce 40-50 rabbit corneal equivalents. One human cornea will provide enough seed culture cells to produce 3-5 human corneal equivalents. No live animals were used in this research project as we obtained the rabbit eyes from a meat processing plant. Human corneal tissue was recovered from post-surgical specimens following corneal transplant surgery. Immunologic techniques were used to develop a reference biomarker database. Interleukin-1 (IL-1) was not detected in the rabbit or human native tissue or the rabbit corneal equivalents. Intercellular adhesion molecule-1 (ICAM-1) and Fibronectin (FN) were inconsistently found in the human and rabbit native tissue and were not detected in the rabbit corneal equivalents. Heat Shock Protein 70 (HSP70) was consistently detected and cataloged in a reference database for both the human and rabbit native tissue and the rabbit corneal equivalents. Rabbit organotypic corneal models demonstrated characteristic histopathologic changes such as coagulative necrosis and liquefactive necrosis following *in vitro* exposure to prototypic chemicals.

14. SUBJECT TERMS	15. NUMBER OF PAGES: 11
Toxicology, In Vitro Methods, Tissue Culture, Rabbits, Human	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT:	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT

19980722 039

DTIC QUALITY INSPECTED 8

REPRODUCTION QUALITY NOTICE

This document is the best quality available. The copy furnished to DTIC contained pages that may have the following quality problems:

- Pages smaller or larger than normal.
- Pages with background color or light colored printing.
- Pages with small type or poor printing; and or
- Pages with continuous tone material or color photographs.

Due to various output media available these conditions may or may not cause poor legibility in the microfiche or hardcopy output you receive.

☑ If this block is checked, the copy furnished to DTIC contained pages with color printing, that when reproduced in Black and White, may change detail of the original copy.

The Development of Corneal Equivalents for In Vitro Testing of Ocular Irritants

AFOSR Grant #F49620-95-1-0168

Final Technical Report (March 1, 1995-April 30, 1998)

Prepared by

Thomas E. Eurell, D.V.M., Ph.D., Dipl. A.B.T.

Department of Veterinary Biosciences

College of Veterinary Medicine

University of Illinois

June 1998

SUMMARY

The principal goal of this study was to produce standardized organotypic models (corneal equivalents) of the rabbit and human cornea using tissue culture methods developed by our research team. Using the methods outlined in this report, one rabbit cornea provided enough seed culture cells to produce 40-50 rabbit organotypic corneal equivalents. Human corneal tissue provided enough seed culture cells to produce 3-5 human organotypic corneal equivalents. No live animals were used in this research project as we obtained the rabbit eyes from a meat processing plant. Human corneal tissue was recovered from post-surgical specimens obtained from a local hospital.

Immunohistochemistry and membrane-based (western blotting of tissue isoelectric focusing profiles) immunologic techniques were used to develop a reference biomarker database The western blotting method proved to be the most reliable procedure to detect biomarker proteins. Interleukin-1 (IL-1) was not detected in the rabbit or human native tissue or the rabbit corneal equivalents. Intercellular adhesion molecule-1 (ICAM-1) and Fibronectin (FN) were inconsistently found in the human and rabbit native tissue and not detected in the rabbit corneal equivalents. Heat Shock Protein 70 (HSP70) was consistently detected and cataloged in a reference database for both the human and rabbit native tissue and the rabbit corneal equivalents.

We did not find any corneal protein biomakers that were consistently useful in differentiating the *in vitro* response of organotypic corneal equivalents to the chemical classes used in this study. However, rabbit organotypic corneal models revealed consistent histopathologic changes such as coagulative necrosis following *in vitro* acidic chemical exposures and liquefactive necrosis following *in vitro* basic chemical exposures. This is important because previous *in vivo* studies in both rabbits and humans have shown that coagulative necrosis occurs with acidic chemicals and liquefactive necrosis occurs with basic chemicals. Thus, the histopathologic response of organotypic models to *in vitro* chemical exposures is similar to the native corneal response following analogous *in vivo* chemical exposures. Furthermore, a Standardized Optical Density (SOD) score for the post-exposure corneal protein profiles was significantly correlated with dose-responsive, degenerative histologic changes in the organotypic corneal equivalents. Our current data suggest the following guidelines for correlating SOD values with the histopathology observed with rabbit organotypic corneal equivalents following chemical exposure:

SOD Values	Histopathologic Tissue Response
≥ 1.20	Control Tissue
1.19-0.9	Minimal Epithelial Change
0.89-0.30	Moderate Epithelial Change/Mild Stromal Change
0.29-0.10	Marked Epithelial Change/Moderate Stromal Change
0.09-0	Severe Epithelial Change/Marked Stromal Change

The occurrence of cytochrome P450 (CYP) enzymes in the rabbit and human native corneas and the rabbit corneal equivalents was an unexpected finding during the course of this project. Using reverse transcriptase-polymerase chain reaction (RT-PCR) methods, the CYP-2b values for control (untreated) native rabbit and human corneas was comparable to what was reported for the rat liver. We believe that our research team is the first to use RT-PCR methods to detect the presence of corneal CYP enzymes. This finding is important because it provides further support for: (1) the similarity between the organotypic corneal equivalents and the native corneas and (2) the extrapolation of organotypic corneal equivalent data to the predicted response of the native corneal tissue. In addition, The presence of biotransformation enzymes such a CYP in the cornea expands the perspective of ocular risk assessment beyond direct irritation or erosion to include the eye as a potential target tissue.

INTRODUCTION

There are currently more than 40 published *in vitro* tests for predicting the ocular irritancy potential of test substances. Although these tests range in complexity from an intricate system using enucleated superfused rabbit eyes to the relatively straightforward growth of established cell lines, they all share the major obstacle of assay validation. Validation of an *in vitro* assay requires that a given endpoint provide a predictable correlation with the *in vivo* response being modeled based on reliable information that is relevant to the decision-making process. This correlation is difficult to attain with many *in vitro* alternatives because they are principally cytotoxicity tests, using target cells or tissues of non-ocular origin which show little resemblance to the complex structure of the eye.

The principal investigator, in collaboration with toxicologists at the Tri-Service Toxicology Laboratory, Wright-Patterson AFB, is studying the application of novel *in vitro* technologies to predict the *in vivo* ocular irritancy response. This effort is focused on the development of organotypic corneal models to address: (1) mechanistic issues regarding *in vitro* alternatives for ocular toxicity, (2) specific proteins found in the cornea, and (3) corneal layer interaction.

The principal objectives of this study were:

(1) To develop three dimensional organotypic models (corneal equivalents) for the rabbit and human cornea

(2) To establish a reference database of corneal proteins and potential biomarkers of corneal toxicity

(3) To establish *in vitro* exposure protocols for the organotypic corneal equivalents to a standardized battery of reference prototype chemicals

This final technical report represents our efforts from March 1, 1995 through April 30, 1998.

USAF Relevancy

The USAF has a scientific mission and a congressional mandate to reduce the number of animals used in research and to protect USAF personnel from operational hazards. The use of *in vitro* methods to screen USAF compounds of interest is an important component of the AFOSR Predictive Toxicology Program (AFOSR Toxicology Program Reviews; 31 May-1 June, 1995 and 12-13 December, 1996, Holiday Inn Conference Center, Fairborn, OH). The transfer of organotypic model technology from our laboratory to the Tri-Service Toxicology Laboratory should provide USAF toxicologists with useful methods that are directly applicable to the AFOSR Predictive Toxicology Program.

Technical Background-Objective 1

Production of three dimensional organotypic corneal equivalents

Explant cultures of corneal epithelial cells and keratocytes were established from native rabbit and human corneal specimens and served as seed cultures for the production of corneal equivalents. Corneal equivalents were produced in two steps using epithelial cells and keratocytes grown from the seed cultures. First, a liquid collagen/corneal keratocytes seed culture suspension was added to a Transwell (Costar) polycarbonate tissue culture insert contained within a 12 well tissue culture plate. The polycarbonate membrane of the insert served as a platform for the gelatinization of the stromal

collagen and the growth of stromal keratocytes. The collagen/ keratocyte suspension formed a gel and the keratocytes were grown in culture for 3-5 days. Second, a seed culture suspension of corneal epithelial cells was plated upon the collagen/ keratocyte gel and grown in culture for an additional 5-7 days. The tissue culture fluid level was slowly lowered over the incubation period until an epithelial cell-air interface was established. The resulting three dimensional models are histologically and biochemically analogous to their respective native tissues (see Results section).

We have had difficulty with the development of human corneal equivalents. Using methods developed by our research team, each rabbit cornea typically provides enough seed culture cells to produce 40-50 organotypic corneal equivalents. The human corneal tissue we receive from the hospital typically provides enough seed culture cells to produce 3-5 organotypic corneal equivalents. We believe there are two reasons for this: (1) the relatively small amounts of human corneal tissue we obtain for a source of seed cell cultures and (2) the difficulty in growing human corneal epithelial cells. We have optimized the growth conditions for the human corneal keratocytes (stromal cells), however, the limiting factor in human corneal equivalent production is epithelial seed cell culture. We plan to continue the development of optimal growth media and conditions for the production of human corneal epithelial cells.

Technical Background-Objective 2

Tissue Isoelectric Focusing (TIF)

Tissue isoelectric focusing was performed following the procedures described by Eurell (Eurell et al., Tox. Appl. Phar. 108:374-378. 1991). One hundred-micron frozen tissue sections were made from control and chemical-exposed corneal equivalents and directly applied to an agarose gel matrix using a mylar template. Electrophoresis was performed in two stages at a constant temperature of 4°C. The samples were focused at 1000 volts, 25 watts and 10 milliamps (current limited) for 15 min. The mylar template and the corneal sections were then removed from the gel surface and the corneal proteins contained within the gel focused for an additional 30 min at 1000 volts, 25 watts, and 350 milliamps (voltage limited). Gels were handled in one of two ways depending upon whether they were analyzed for biomarker proteins [Heat Shock Protein 70 (HSP70); Interleukin-1 (IL-1); Intercellular adhesion molecule-1 (ICAM-1); Fibronectin (FN)] or total protein profile density.

Detection of Potential Biomarker Proteins

Immunologic detection of potential biomarker proteins (HSP70, IL-1, ICAM-1 and FN) in TIF protein profiles was accomplished in three steps. First, the proteins recovered by the tissue isoelectric focusing procedure were transferred to a nitrocellulose membrane (western blotting electrophoresis). Second, the nitrocellulose membrane was incubated with specific antibodies against the four potential biomarker proteins (anti-HSP70-StressGen, Inc.; anti-IL-1a-Chemicon, Inc.; anti-ICAM-1-Chemicon, Inc.; anti-FN-Calbiochem, Inc.) and the antigen-antibody reaction detected using an alkaline phosphatase-nitroblue tetrazolium reporter system (Sigma). Third, the nitroblue tetrazolium-stained protein bands were scanned using a scanning densitometer and the final biomarker concentrations determined using biomarker-specific standard curves and computer-based integration (ChemResearch, Isco).

Gels to be analyzed for protein profile density were fixed and stained according to the methods described by Eurell (Eurell et al., Tox. Appl. Phar. 108:374-378. 1991). The processed gels were stained using a commercial silver stain kit for agarose (Isolab). The isoelectric point and relative density of

individual corneal protein bands as well as the overall density of the protein profile was determined using a scanning densitometer. Integration and comparison of densitometric data was performed using the ChemResearch Program (Isco).

Technical Background-Objective 3

In Vitro Exposure of Organotypic Corneal Equivalents to Prototypic Test Chemicals

A review of the literature and the ECETOC Eye Irritation Reference Chemicals Data Bank was used to select the following test compounds: ethylene glycol, Tween-20, ethanol, acetone, n-butanol, acetic acid and sodium hydoxide. Depending upon the dose, ethylene glycol and Tween-20 are considered mild ocular irritants, sodium dodecyl sulfate, ethanol and acetone are considered mild to moderate ocular irritants, n-butanol, acetic acid and sodium hydroxide are considered strong ocular irritants. Test compounds were diluted in culture media and applied in a volume of 10 microliters to the epithelial surface of the corneal equivalents for 10 min, at room temperature. Based on preliminary studies, three dose concentrations of each test compound were used. The corneal equivalents were washed 3 times in tissue culture media and then returned to the tissue culture incubator for an overnight post-exposure incubation. Following the post-exposure period tissue culture fluid overlying the corneal equivalents was harvested and the tissue quick frozen in liquid nitrogen and stored at -70^{o}C until processed for TIF. Post-exposure alterations in the histologic appearance and biomarker profiles of the corneal equivalents were compared to a reference database developed in our laboratory.

RESULTS

Objective I Develop three-dimensional organotypic models (corneal equivalents) for the rabbit and human cornea

The rabbit anterior cornea consists of a stratified non-keratinized epithelium and a stromal matrix (Fig 1A). The corneal epithelium is composed of 4 to 6 layers and is divided into basal, wing and superficial cells by cell morphology and location. The basal cell layer is 1 cell thick and the wing and superficial cell layers are each 2 to 3 cells thick. The basal cell layer serves as the source for both the cells in the outer layers of the corneal epithelium and the epithelial basement membrane. The corneal stroma is a complex matrix of collagen fibers, keratocytes and glycosaminoglycans. The collagen fibers are arranged in lamellar sheets that are oriented in a plane parallel to the epithelial surface. Keratocytes and glycosaminoglycans are located between the lamellar sheets of collagen.

The rabbit and human corneal equivalents were grown *in vitro* and produced through the co-culture of epithelial and stromal cells from corneal tissues of their respective species. The histologic appearance of the corneal equivalents was similar to the native tissues with the characteristic differentiation and stratification of the epithelial layer into basal, wing and superficial cells (Fig 1B). A prominent basement membrane was secreted by the cells in the basal layer of the corneal equivalents. The stromal collagen matrix of the corneal equivalents was more homogenous in appearance than the analogous structure in the native tissue. Preliminary studies suggest that this was due to a more structured organization of the collagen fibers in the native tissue. We have recently been able to optimize the culture conditions of the corneal keratocytes to induce the lamellar orientation of the collagen fibers (Fig 4A).

Figure 1(A). Histologic section of native rabbit cornea. (A) Stratified corneal epithelium composed of a superficial layer (A1), 2-3 layers of intermediate or wing cells (A2) and a single layer of basal cells (A3). (B) Stromal matrix, note fusiform stromal keratocyte (arrow). Formalin-fixed, paraffin-embedded specimen, H&E stain, original magnification=400x.

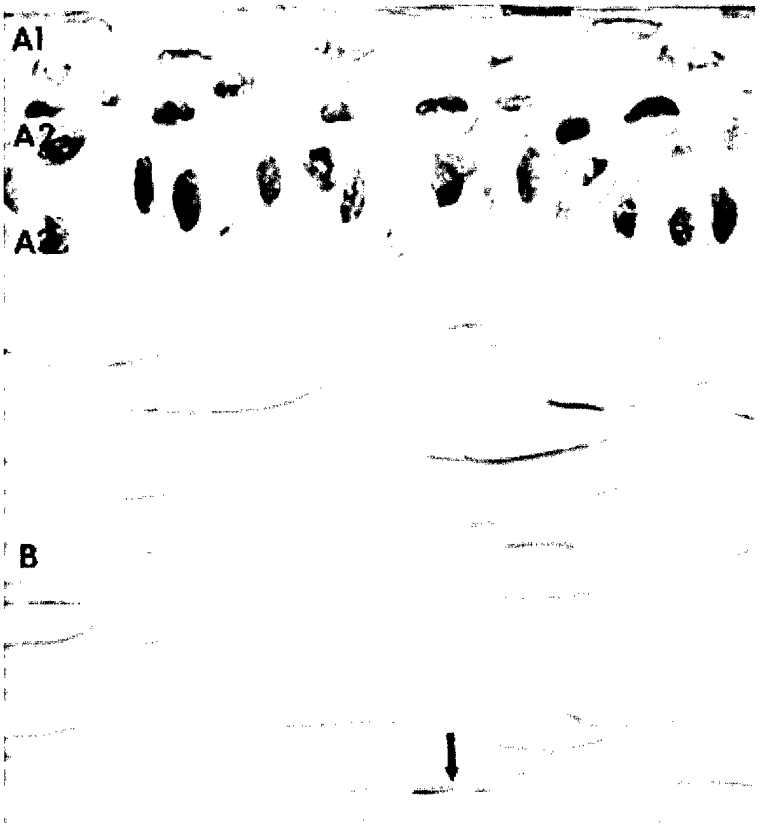
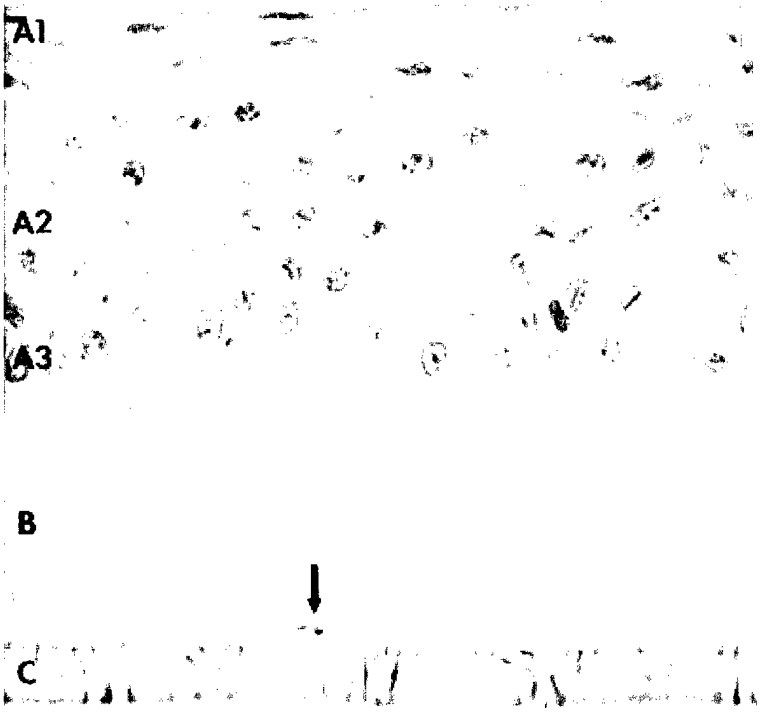


Figure 1(B). Histologic section of rabbit corneal equivalent. (A) Stratified corneal epithelium composed of a superficial layer (A1), 4-5 layers of intermediate or wing cells (A2) and a single layer of basal cells (A3). (B) Stromal matrix, note fusiform stromal keratocyte (arrow). (C) Polycarbonate supporting membrane. Formalin-fixed, paraffin-embedded specimen, H&E stain, original magnification=400x.



Objective 2 Establish a database of corneal proteins and potential biomarkers of corneal toxicity

Reference corneal proteins

Isoelectric focusing of tissue sections from human and rabbit native corneal specimens resulted in a species-specific TIF protein profile (Fig 2). The rabbit native cornea had a consistent TIF protein profile where most of the individual protein bands occurred in the acidic range (pI< 7.0). Native human corneal TIF protein profiles were less consistent and more complex than the analogous rabbit profiles. The human corneal TIF pattern had a higher concentration (protein band density) of proteins with a pI >7.0. Comparison of reference TIF protein profiles from a native rabbit cornea (Fig 2, Lane C) and a rabbit corneal equivalent (Fig 2, Lane D) reveal that the corneal equivalent has a protein profile almost identical to the native tissue.

Potential Corneal Biomarkers of Toxicity

Interleukin-1 (IL-1), Intercellular adhesion molecule-1 (ICAM-1), Fibronectin (FN) and Heat Shock Protein 70 (HSP70) were chosen from the literature as potential corneal biomarkers. Immunohistochemistry and membrane-based (western blotting of tissue isoelectric focusing profiles) immunologic techniques were used to develop the reference biomarker database for the corneal tissue. The western blotting method proved to be the most reliable procedure to detect biomarker proteins. Interleukin-1 (IL-1) was not detected in the rabbit or human native tissue or the rabbit corneal equivalents. Intercellular adhesion molecule-1 (ICAM-1) and Fibronectin (FN) were inconsistently found in the human and rabbit native tissue and were not detected in the rabbit corneal equivalents. Heat Shock Protein 70 (HSP70) was consistently detected (Fig 3) and cataloged in a reference database developed for both the human and rabbit native tissue and the rabbit corneal equivalents

Objective 3 Establish *in vitro* exposure protocols for the organotypic corneal equivalents to a standardized battery of reference prototype chemicals

Although HSP70 was consistently detected in the corneal tissue, it was not useful in differentiating the *in vitro* response of organotypic corneal equivalents to the chemical classes used in this study. However, a standardized optical density score was very useful in differentiating the post-exposure *in vitro* response of organotypic corneal equivalents (Fig 4). We use the term Standardized Optical Density (SOD) to refer to calibrated digital images of the TIF data. All TIF gels contain an internal standard protein lane for calibration of test sample data. This assures reproducibility across each experiment. Calibrated test sample data is then standardized against an external calibrated optical density scale (Kodak) to arrive at the SOD.

The *in vivo* exposure of both rabbit and human tissue to acids typically causes coagulative necrosis, whereas, *in vivo* exposure to bases typically causes liquefactive necrosis. Characteristic features of coagulative and liquefactive necrosis were observed in the corneal equivalents following chemical exposure (Fig 5). In coagulative necrosis, the normal architecture of the tissue and its cellular components is still recognizable, however, the nulcei exhibit characteristic changes including pyknosis and karyorrhexis (Fig 5B). In liquefactive necrosis, the normal architecture of the tissue and its cellular components are often not recognizable and there usually is a characteristic loss of nuclear detail (Fig 5C). Thus, the histopathologic response of organotypic models to *in vitro* chemical exposures is similar to the native corneal response following analogous *in vivo* chemical exposures. Furthermore, SOD scores for the post-exposure corneal protein profiles were significantly correlated with dose-responsive, degenerative histologic changes in the organotypic corneal equivalents.

Figure 2. Tissue isoelectric focusing protein profiles from representative sections of human and rabbit corneas and a rabbit corneal equivalent. Lane A=isoelectric focusing standards (1=pH 5.1, 2=pH 6.0, 3=pH 7.2 and 4=pH 8.2). Lane B=human corneal section. Lane C=rabbit corneal section. Lane D=rabbit corneal equivalent section. One hundred-micron sections were made from human and rabbit corneal specimens and a rabbit corneal equivalent, processed by tissue isoelectric focusing and the resulting protein bands were stained using a silver stain. Comparison of reference TIF protein profiles from a native rabbit cornea (Lane C) and a rabbit corneal equivalent (Lane D) reveal that the corneal equivalent has a protein profile almost identical to the native tissue.

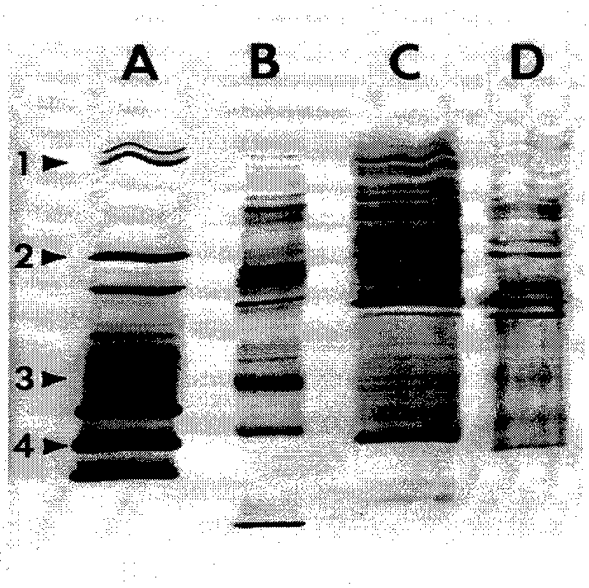


Figure 3. Protein profiles from rabbit corneal equivalents after acute exposure to 0.1 N and 0.2 N concentrations of acetic acid. Lanes A (0.1) + B (0.2) are from isoelectric focusing gels that were silver stained to show the total protein profiles of exposed corneal equivalents. Lanes C + D are from isoelectric focusing gels of the samples run in Lanes A + B, respectively that were then reacted against antibody specific for HSP70. The arrow indicates the relative position of HSP70 in both the silver stained preparations and the western blots. Note that the relative concentration (stain intensity) of HSP70 decreases with increasing concentration of acetic acid

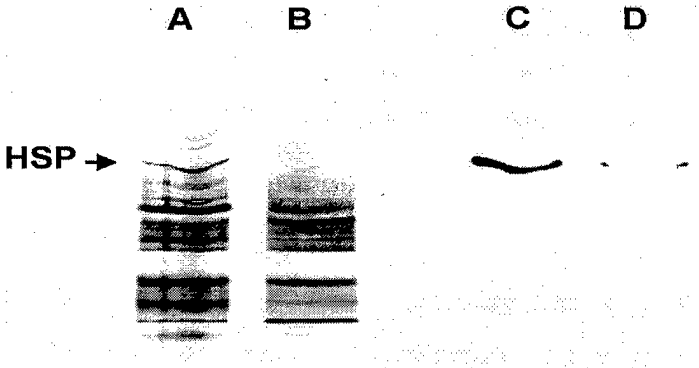


Figure 4. Representative protein profiles from rabbit corneal equivalents after acute exposure to increasing concentrations of sodium hydroxide. Lane A=0.006 N sodium hydroxide. Lanes B-F=0.012, 0.025, 0.05, 0.1 and 0.2 N sodium hydroxide, respectively. Compare the protein profile in Lane F (0.2 N) with the matching histopathologic changes in Figure 5 (C). Standardized Optical Density scores for Lanes A-F= 1.10, 0.88, 0.52, 0.28, 0.10 and 0.06, respectively.

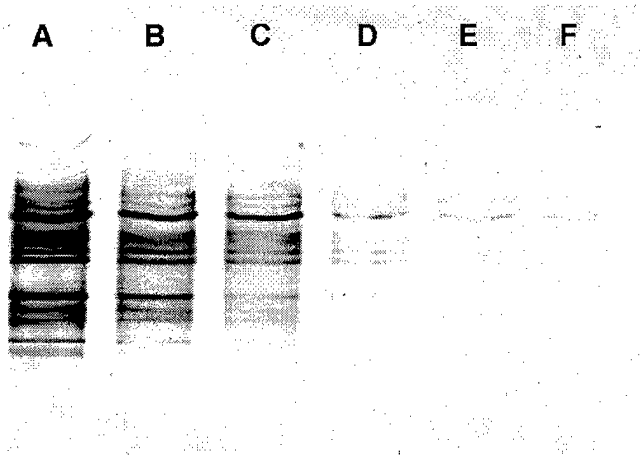


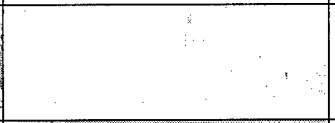
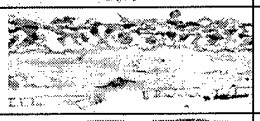
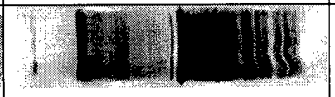
Figure 5. Representative digital images from frozen sections of rabbit corneal equivalents. (A.) Control corneal equivalent. (→) indicates transition from stroma (below arrow) to epithelial layer (above arrow). Note lamellar organization of collagen fibers. (B.) Corneal equivalent exposed to 0.2 N acetic acid. Note alterations in architecture of epithelial cells characterized by pyknosis (↑) and karyorrhexis ( ). (C.) Corneal equivalent exposed to 0.2 N sodium hydroxide. Note general loss of cellular detail in the epithelial layer. (Original magnification = 400X; * indicates polycarbonate support membrane.)

SUMMARY OF ACCOMPLISHMENTS AND SIGNIFICANCE OF RESEARCH PROJECT

1. Development of Organotypic Corneal Equivalents- Figure 1(B) represents our initial development of the rabbit organotypic model at the beginning of this research project. We were concerned by the lack of collagen lamellae in the stromal matrix of the corneal equivalents (compare Fig 1(A) Layer B with Fig 1(B) Layer B). Within the last year we have made significant progress in the development of rabbit organotypic corneal equivalents and have optimized the stromal matrix culture conditions to induce the formation of collagen lamella as demonstrated in Figure 5A. This is an important accomplishment because of the complex stromal-epithelial layer interactions observed with the *in vivo* ocular irritancy response. We believe that we are currently the only research team in the country to accomplish this goal.

2. Standardized Optical Density Measurements of TIF Data- The advantage of this approach is that any laboratory using a scanner calibrated with the commercially available Kodak optical density scale should be able to reproduce our data. This will allow direct technology transfer from our laboratory to AFOSR laboratories.

3. Development of a Prototypic Chemical Database for Ocular Irritants- The calibrated optical density data for TIF protein profiles in conjunction with the archived histopathology images for each exposure will provide a useful database for chemical evaluation in the AFOSR Predictive Toxicology Program. The database is in Microsoft Word/Excel/Access format for easy technology transfer and is represented in the following table.

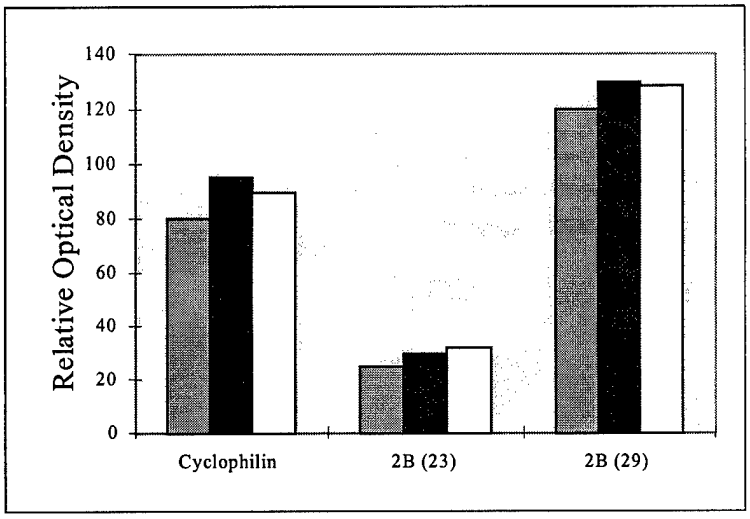
Chemical Agent	Histopathology	TIF Protein Profile	Standardized Optical Density
0.2 N Sodium Hydroxide Severe Irritant			0.06
0.2 N Acetic Acid Moderate Irritant			0.84
1.0 % Ethylene Glycol Mild Irritant			1.12

4. Correlation between histopathology and TIF- Originally, we were looking for changes in potential biomarker proteins (e.g., IL-1, ICAM-1, FN, HSP70) that were selected after an extensive literature review. This approach did not correlate well with the tissue response following exposure to our battery of prototypic chemicals. We believe that a much better correlation exists between the Standardized Optical Density (SOD) of the TIF protein profile and the histopathologic changes associated with chemical exposure. Our current data suggest the following guidelines for correlating SOD values with the histopathology observed with rabbit organotypic corneal equivalents following chemical exposure:

SOD Values	Histopathologic Tissue Response
≥ 1.20	Control Tissue
1.19-0.9	Minimal Epithelial Change
0.89-0.30	Moderate Epithelial Change/Mild Stromal Change
0.29-0.10	Marked Epithelial Change/Moderate Stromal Change
0.09-0	Severe Epithelial Change/Marked Stromal Change

5. Corneal Cytochrome P450- The occurrence of cytochrome P450 (CYP) enzymes in the rabbit and human native corneas and the rabbit corneal equivalents was an unexpected finding during the course of this project. This finding is important because it provides further support for: (1) the similarity between the organotypic corneal equivalents and the native corneas and (2) the extrapolation of organotypic corneal equivalent data to the predicted response of the native corneal tissue. In addition, The presence of biotransformation enzymes such a CYP in the cornea expands the perspective of ocular risk assessment beyond direct irritation or erosion to include the eye as a potential target tissue.

RT-PCR analysis of CYP2B-a rabbit native corneal cytochrome P450 enzyme. Total corneal RNA was isolated from 3 control rabbit corneas and analyzed for the expression of CYP2B using RT-PCR. PCR products were separated on 5% native PAGE gels, stained with ethidium bromide, and the relative optical density determined using scanning densitometry. The housekeeping gene, cyclophilin, was used as a loading control. 2B (23) = 23 cycles amplification. 2B (29) = 29 cycles amplification. Note: The CYP2B values for the control (untreated) native rabbit corneas are comparable to what is reported for the rat liver.



PUBLICATIONS FROM RESEARCH PROJECT

GD Meyer, TE Eurell, DR Mattie, and DJ Caldwell. The *in vitro* ocular irritancy of liquid propellant XM46 using native rabbit corneas and organotypic corneal equivalents. The Toxicologist Vol. 30 (1), p 124, 1996.

TE Eurell and DR Mattie. Histologic and biochemical characteristics of rabbit corneal equivalents. Manuscript in preparation.

INTERACTIONS (COUPLING ACTIVITIES) AND TRANSITIONS

Meetings

AFOSR Research Reviews- May 31-June 1, 1995 and December 11-13, 1996- held at the Holiday Inn, Dayton, OH.

Society of Toxicology Meetings, March 10-14, 1996, Anaheim, CA and March 1-5, 1998, Seattle, WA.

Consultation

June 1, 1995-reviewed research progress with Drs. D. Mattie and D. Caldwell (Tri-Service Toxicology)

January 12, March 10, and July 8, 1996-reviewed research progress with Dr. D. Mattie

March 14, and July 25, 1997-reviewed research progress with Dr. D. Mattie

March 1, 1998-reviewed research progress with Dr. D. Mattie

Transitions

Conducted and reported on *in vitro* toxicity of XM46 developmental liquid propellant for Tri-Service Toxicology Laboratory, Wright Patterson AFB.

Dr. Gary Meyer (Major, U.S. Air Force) completed his Ph.D. research in my laboratory this summer and is currently a staff scientist in the toxicology lab at Wright-Patterson AFB. Dr. Meyer brings a full complement of our research methods and will play an important role in the technology transfer to the Tri-Service Toxicology Laboratory

HONORS AND AWARDS

Dr. Eurell received the Merck AgVet Award for Creativity, University of Illinois on August 4, 1995.

Dr. Eurell received a Colgate-Palmolive/Society of Toxicology Award on March 13, 1996.